

CLAIMS

We claim:

1. A method for amplification of a template polynucleotide, comprising:
 - (a) incubating a reaction mixture, said reaction mixture comprising:
 - (i) a template polynucleotide;
 - (ii) a first primer, wherein the first primer is a composite primer that is hybridizable to a multiplicity of template polynucleotide sites, wherein the composite primer comprises an RNA portion and a 3' DNA portion;
 - (iii) a DNA-dependent DNA polymerase; and
 - (iv) an RNA-dependent DNA polymerase;wherein the incubation is under conditions that permit first primer random hybridization to the template polynucleotide, and primer extension, whereby a complex comprising a RNA/DNA heteroduplex is generated; and
 - (b) incubating a reaction mixture, said reaction mixture comprising
 - (i) at least a portion of the reaction products generated according to step (a);
 - (ii) an amplification primer, wherein said amplification primer is a composite primer comprising an RNA portion and a 3' DNA portion;
 - (iii) an DNA-dependent DNA polymerase; and
 - (iv) an agent that cleaves RNA from an RNA/DNA hybrid;wherein the incubation is under conditions that permit RNA cleavage, primer hybridization, primer extension, and displacement of the primer extension product when its RNA is cleaved and another amplification primer binds to the template and is extended by strand displacement, whereby multiple copies of a polynucleotide amplification product are generated.
2. The method of claim 1, wherein said DNA-dependent DNA polymerase said RNA-dependent DNA polymerase of step (a) are the same enzyme.
3. The method of claim 1, wherein said DNA-dependent DNA polymerase said RNA-dependent DNA polymerase of step (a) are different enzymes.

4. The method of claim 1, wherein said first primer and said amplification primer are the same primer.
5. The method of claim 1, wherein said first primer and said amplification primer are different primers.
6. The method of claim 1, wherein step (b) is initiated by the addition of an agent that cleaves RNA from an RNA/DNA heteroduplex to the reaction mixture of step (a).
7. The method of claim 6, wherein said agent that cleaves RNA from an RNA/DNA heteroduplex is RNase H.
8. The method of claim 1, wherein the reaction mixture of step (a) further comprises auxiliary primers.
9. The method of claim 8, wherein the reaction mixture of step (b) further comprises auxiliary primers.
10. The method of claim 1, wherein the reaction mixture of step (b) further comprises auxiliary primers.
11. The method of claim 1, wherein said template polynucleotide is DNA.
12. The method of claim 11, wherein said first primer comprises a random sequence.
13. The method of claim 12, wherein said first primer is at least two different primers.
14. The method of claim 13, wherein said first primer is a plurality of different primers.
15. The method of claim 1, wherein said template polynucleotide is RNA.
16. The method of claim 15, wherein said template RNA is mRNA.
17. The method of claim 15, wherein the reaction mixture of step (a) further comprises an agent that cleaves RNA from a RNA/DNA heteroduplex to the reaction mixture of step (a).
18. The method of claim 17, wherein the agent in the reaction mixture of step (a) that cleaves RNA in a RNA/DNA heteroduplex is an enzyme.
19. The method of claim 18, wherein the enzyme that cleaves RNA in a RNA/DNA heteroduplex in the reaction mixture of step (a) is RNase H.

20. The method of claim 17, wherein said first primer and said amplification primer are different primers.
21. The method of claim 20, wherein step (b) is initiated by addition of the amplification primer to the reaction mixture of step (a).
22. The method of claim 17, wherein said DNA-dependent DNA polymerase said RNA-dependent DNA polymerase of step (a) are the same enzyme.
23. The method of claim 17, wherein said DNA-dependent DNA polymerase said RNA-dependent DNA polymerase of step (a) are different enzymes.
24. The method of claim 17, wherein the agent that cleaves RNA from an RNA/DNA heteroduplex is RNase H.
25. The method of claim 17, wherein the reaction mixture of step (a) further comprises auxiliary primers.
26. The method of claim 17, wherein the reaction mixture of step (b) further comprises auxiliary primers.
27. The method of claim 1, wherein the RNA portion of the first primer is 5' with respect to the 3'-DNA portion.
28. The method of claim 27, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.
29. The method of claim 28, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.
30. The method of claim 27, wherein the 5' RNA portion of the first primer is adjacent to the 3' DNA portion.
31. The method of claim 30, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.
32. The method of claim 31, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.
33. The method of claim 1, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.
34. The method of claim 33, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.

35. The method of claim 1, wherein the RNA portion of the first primer consists of 7 to about 20 nucleotides.
36. The method of claim 35, wherein the DNA portion of the first primer consists of about 5 to about 20 nucleotides.
37. The method of claim 36, wherein the RNA portion of the first primer consists of about 10 to about 20 nucleotides.
38. The method of claim 37, wherein the DNA portion of the first primer consists of about 7 to about 20 nucleotides.
39. The method of claim 1, wherein the RNA portion of the amplification primer consists of 7 to about 20 nucleotides.
40. The method of claim 39, wherein the DNA portion of the amplification primer consists of about 5 to about 20 nucleotides.
41. The method of claim 40, wherein the RNA portion of the amplification primer consists of about 10 to about 20 nucleotides.
42. The method of claim 41, wherein the DNA portion of the amplification primer consists of about 7 to about 20 nucleotides.
43. The method of claim 1, wherein the first primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.
44. The method of claim 43, wherein the amplification primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.
45. The method of claim 1, wherein the amplification primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.
46. The method of claim 1, wherein the reaction mixture of step (b) further comprises a non-canonical nucleotide.

47. The method of claim 46, wherein the non-canonical nucleotide is dUTP.

48. The method of claim 1, wherein the reaction mixture of step (b) further comprises a labeled nucleotide.

49. A method for amplification of a template polynucleotide, comprising:
incubating a reaction mixture, said reaction mixture comprising:

(a) a complex comprising a RNA/DNA partial heteroduplex, wherein the complex is generated by incubating a first reaction mixture, said first reaction mixture comprising:

(i) a polynucleotide template;

(ii) a first primer; wherein the first primer is a composite primer, the composite primer comprises an RNA portion and a 3' DNA portion; and wherein the composite primer is capable of hybridizing to a multiplicity of template polynucleotide sites;

(iii) a DNA-dependent DNA polymerase; and

(iv) an RNA-dependent DNA polymerase;

wherein the incubation is under conditions that permit first primer random hybridization, and primer extension, whereby a complex comprising an RNA/DNA partial heteroduplex is generated;

(b) an amplification primer, wherein the amplification primer is a composite primer comprising an RNA portion and a 3' DNA portion;

(c) a DNA-dependent DNA polymerase; and

(d) an agent that cleaves RNA from an RNA/DNA hybrid;

wherein the incubation is under conditions that permit RNA cleavage, primer hybridization, primer extension, and displacement of primer extension product when its RNA is cleaved and another amplification primer binds and is extended, whereby multiple copies of a polynucleotide amplification product are generated.

50. The method of claim 1, wherein the reaction mixture further comprises auxiliary primers.

51. The method of claim 1, wherein the agent that cleaves RNA from an RNA/DNA hybrid is an enzyme.

52. The method of claim 51, wherein the enzyme that cleaves RNA from an RNA/DNA hybrid is RNase H.
53. The method of claim 51, wherein said DNA-dependent DNA polymerase and said enzyme that cleaves RNA from an RNA/DNA hybrid are the same enzyme.
54. The method of claim 53, wherein said DNA-dependent DNA polymerase and said enzyme that cleaves RNA from an RNA/DNA hybrid are different enzymes.
55. The method of claim 49, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.
56. The method of claim 55, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.
57. The method of claim 49, wherein the RNA portion of the amplification primer consists of 7 to about 20 nucleotides.
58. The method of claim 57, wherein the DNA portion of the amplification primer consists of about 5 to about 20 nucleotides.
59. The method of claim 58, wherein the RNA portion of the amplification primer consists of about 10 to about 20 nucleotides.
60. The method of claim 59, wherein the DNA portion of the amplification primer consists of about 7 to about 20 nucleotides.
61. The method of claim 49, wherein the amplification primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.
62. The method of claim 49, wherein the reaction mixture further comprises a non-canonical nucleotide.
63. The method of claim 62, wherein the non-canonical nucleotide is dUTP.
64. The method of claim 49, wherein the reaction mixture further comprises a labeled nucleotide.

65. A method for amplification of a template polynucleotide, comprising:
incubating a reaction mixture, said reaction mixture comprising:

(a) a complex of a first primer extension product and a second primer extension product, wherein the first primer extension product is generated by extension of a randomly primed first primer hybridized to target polynucleotide with a DNA polymerase, wherein the first primer is a composite primer comprising an RNA portion and a 3' DNA portion, wherein the first primer is capable of hybridizing to a multiplicity of template polynucleotide sites, and wherein the second primer extension product is generated by extension of a second primer hybridized to the first primer extension product;

(b) an amplification primer, wherein the amplification is a composite primer comprises an RNA portion and a 3' DNA portion and is hybridizable to the second primer extension product;

(c) a DNA-dependent DNA polymerase; and

(d) an agent that cleaves RNA from an RNA/DNA hybrid;

wherein the incubation is under conditions that permit RNA cleavage, primer hybridization, primer extension, and displacement of composite primer extension product from the second primer extension product when the RNA portion of the composite primer is cleaved and another composite primer binds and is extended, whereby multiple copies of a polynucleotide amplification product are generated.

66. The method of claim 65, wherein said reaction mixture further comprises auxiliary primers.

67. The method of claim 65, wherein said agent that cleaves RNA from a RNA/DNA hybrid is an enzyme.

68. The method of claim 67, wherein the enzyme that cleaves RNA from a RNA/DNA hybrid RNase H.

69. The method of claim 67, wherein said DNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA hybrid are the same enzyme.

70. The method of claim 65, wherein said DNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA hybrid are different enzymes.
71. The method of claim 65, wherein said template polynucleotide is RNA.
72. The method of claim 65, wherein said template polynucleotide is DNA.
73. The method of claim 65, wherein said reaction mixture further comprises auxiliary primers.
74. The method of claim 65, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.
75. The method of claim 74, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.
76. The method of claim 65, wherein the RNA portion of the amplification primer consists of 7 to about 20 nucleotides.
77. The method of claim 76, wherein the DNA portion of the amplification primer consists of about 5 to about 20 nucleotides.
78. The method of claim 77, wherein the RNA portion of the amplification primer consists of about 10 to about 20 nucleotides.
79. The method of claim 78, wherein the DNA portion of the amplification primer consists of about 7 to about 20 nucleotides.
80. The method of claim 65, wherein the amplification primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.
81. The method of claim 65, wherein the reaction mixture further comprises a non-canonical nucleotide.
82. The method of claim 81, wherein the non-canonical nucleotide is dUTP.
83. The method of claim 65, wherein the reaction mixture further comprises a labeled nucleotide.

84. A method for amplification of a polynucleotide template, comprising:
(a) random priming of polynucleotide template strand with a composite primer; wherein the composite primer comprises an RNA portion and a 3' DNA portion, and wherein the composite primer is capable of hybridizing to a multiplicity of template polynucleotide sites, thereby producing a complex comprising a composite primer randomly hybridized to polynucleotide template; and

(b) incubating the complex in the presence of a DNA-dependent DNA polymerase, an RNA-dependent DNA polymerase, and an agent that cleaves RNA from a RNA/DNA heteroduplex, whereby multiple copies of polynucleotide amplification product are generated by primer extension and strand displacement.

85. The method of claim 84, wherein step (a) further comprises auxiliary primers.

86. The method of claim 85, wherein step (b) further comprises auxiliary primers.

87. The method of claim 84, wherein step (b) further comprises auxiliary primers.

88. The method of claim 84, wherein step (a) further comprises a DNA-dependent DNA polymerase.

89. The method of claim 88, wherein step (a) further comprises a RNA-dependent DNA polymerase.

90. The method of claim 84, wherein the agent that cleaves RNA from a RNA/DNA heteroduplex is an enzyme.

91. The method of claim 90, wherein the enzyme that cleaves RNA from a RNA/DNA heteroduplex is RNase H.

92. The method of claim 90, wherein the RNA-dependent DNA polymerase, and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are the same enzyme.

93. The method of claim 92, wherein the DNA-dependent DNA polymerase, the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are the same enzyme.

94. The method of claim 90, wherein the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are different enzymes.
95. The method of claim 94, wherein the DNA-dependent DNA polymerase, the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are all different enzymes.
96. The method of claim 84, wherein the DNA-dependent DNA polymerase and the RNA-dependent DNA polymerase are the same enzyme.
97. The method of claim 84, wherein the DNA-dependent DNA polymerase and the RNA-dependent DNA polymerase are different enzymes.
98. The method of claim 84, wherein the RNA portion of the composite primer is 5' with respect to the 3'-DNA portion.
99. The method of claim 98, wherein the 5' RNA portion of the composite primer is adjacent to the 3' DNA portion.
100. The method of claim 84, wherein the RNA portion of the composite primer consists of 7 to about 20 nucleotides.
101. The method of claim 100, wherein the DNA portion of the composite primer consists of about 5 to about 20 nucleotides.
102. The method of claim 101, wherein the RNA portion of the composite primer consists of about 10 to about 20 nucleotides.
103. The method of claim 102, wherein the DNA portion of the composite primer consists of about 7 to about 20 nucleotides.
104. The method of claim 84, wherein the composite primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.
105. The method of claim 84, wherein the step of incubating is further in the presence of a non-canonical nucleotide.
106. The method of claim 105, wherein the non-canonical nucleotide is dUTP.

107. The method of claim 84, wherein the step of incubating is further in the presence of a labeled nucleotide.

108. A method for amplification of a polynucleotide template, comprising:

(a) randomly priming a template polynucleotide with a first primer, wherein said first primer is a composite primer that is hybridizable to a multiplicity of template polynucleotide sites, wherein the composite primer comprises a RNA portion and a 3' DNA portion;

(b) extending said first primer with a DNA polymerase;

(c) cleaving RNA from the first primer with an agent that cleaves RNA from a RNA/DNA heteroduplex;

(d) hybridizing an amplification primer to the template polynucleotide, wherein said amplification primer is a composite primer comprising a RNA portion and a 3' DNA portion;

(e) extending the hybridized amplification primer by strand displacement DNA synthesis;

(f) cleaving RNA from the amplification primer with an agent that cleaves RNA from a RNA/DNA heteroduplex, such that another amplification primer can hybridize and be extended,

whereby multiple copies of a polynucleotide amplification product are generated.

109. The method of claim 108, wherein said template polynucleotide is DNA.

110. The method of claim 109, wherein said first primer comprises a random sequence.

111. The method of claim 110, wherein said first primer is at least two primers.

112. The method of claim 111, wherein said first primer is a plurality of primers.

113. The method of claim 108, wherein said template polynucleotide is RNA.

114. The method of claim 113, further comprising cleaving the template polynucleotide with an agent that cleaves RNA in a RNA/DNA hybrid.
115. The method of claim 114, wherein the agent that cleaves RNA in a RNA/DNA hybrid is an enzyme.
116. The method of claim 115, wherein the enzyme that cleaves RNA in a RNA/DNA hybrid is RNase H.
117. The method of claim 115, wherein the first primer is extended with a RNA-dependent DNA polymerase.
118. The method of claim 113, wherein the first primer is extended with a RNA-dependent DNA polymerase.
119. The method of claim 108, comprising repeating steps (d) through (f).
120. The method of claim 108, wherein the DNA polymerase of step (b) is a DNA-dependent DNA polymerase.
121. The method of claim 120, wherein the first primer is also extended with a RNA-dependent DNA polymerase.
122. The method of claim 121, wherein the DNA-dependent DNA polymerase and the RNA-dependent DNA polymerase are the same enzyme.
123. The method of claim 122, wherein the DNA-dependent DNA polymerase and the RNA-dependent DNA polymerase are different enzymes.
124. The method of claim 108, wherein said first primer and said amplification primer are the same primer.
125. The method of claim 108, wherein said first primer and said amplification primer are different primers.
126. The method of claim 108, wherein step (a) further comprises hybridizing auxiliary primers to the template polynucleotide.
127. The method of claim 108, wherein the RNA portion of the first primer is 5' with respect to the 3'-DNA portion.
128. The method of claim 127, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.
129. The method of claim 128, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.

130. The method of claim 127, wherein the 5' RNA portion of the first primer is adjacent to the 3' DNA portion.
131. The method of claim 130, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.
132. The method of claim 131, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.
133. The method of claim 108, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.
134. The method of claim 133, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.
135. The method of claim 108, wherein the RNA portion of the first primer consists of 7 to about 20 nucleotides.
136. The method of claim 135, wherein the DNA portion of the first primer consists of about 5 to about 20 nucleotides.
137. The method of claim 136, wherein the RNA portion of the first primer consists of about 10 to about 20 nucleotides.
138. The method of claim 137, wherein the DNA portion of the composite primer consists of about 7 to about 20 nucleotides.
139. The method of claim 108, wherein the RNA portion of the first primer consists of 7 to about 20 nucleotides.
140. The method of claim 139, wherein the DNA portion of the first primer consists of about 5 to about 20 nucleotides.
141. The method of claim 140, wherein the RNA portion of the first primer consists of about 10 to about 20 nucleotides.
142. The method of claim 141, wherein the DNA portion of the composite primer consists of about 7 to about 20 nucleotides.
143. The method of claim 108, wherein the first primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.

144. The method of claim 143, wherein the amplification primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.

145. The method of claim 108, wherein the amplification primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.

146. The method of claim 108, wherein step (e) is carried out in the presence of a non-canonical nucleotide.

147. The method of claim 146, wherein the non-canonical nucleotide is dUTP.

148. The method of claim 108, wherein step (e) is carried out in the presence of a labeled nucleotide.

149. A method for amplification of a polynucleotide template, comprising: incubating a reaction mixture comprising:

(a) a polynucleotide template strand;

(b) a first primer, wherein said first primer is a composite primer comprising a RNA portion and a 3' DNA portion, and wherein the first primer is capable of hybridizing to a multiplicity of template polynucleotide sites;

(c) a DNA-dependent DNA polymerase;

(d) a RNA-dependent DNA polymerase; and

(e) an agent that cleaves RNA from a RNA/DNA heteroduplex,

whereby multiple copies of polynucleotide amplification product are generated by primer extension and strand displacement.

150. The method of claim 149, wherein the reaction mixture further comprises auxiliary primers.

151. The method of claim 149, wherein the agent that cleaves RNA from a RNA/DNA heteroduplex is an enzyme.

152. The method of claim 151, wherein the enzyme that cleaves RNA from a RNA/DNA heteroduplex is RNase H.

153. The method of claim 151, wherein the RNA-dependent DNA polymerase, and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are the same enzyme.

154. The method of claim 153, wherein the DNA-dependent DNA polymerase, the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are the same enzyme.

155. The method of claim 151, wherein the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are different enzymes.

156. The method of claim 155, wherein the DNA-dependent DNA polymerase, the RNA-dependent DNA polymerase and the enzyme that cleaves RNA from an RNA/DNA heteroduplex are all different enzymes.

157. The method of claim 149, wherein the DNA-dependent DNA polymerase and the RNA-dependent DNA polymerase are the same enzyme.

158. The method of claim 149, wherein the DNA-dependent DNA polymerase and the RNA-dependent DNA polymerase are different enzymes.

159. The method of claim 149, wherein said reaction mixture further comprises an amplification primer, wherein said amplification primer is a composite primer comprising a RNA portion and a 3' DNA portion.

160. The method of claim 159, wherein the RNA portion of the amplification primer is 5' with respect to the 3'-DNA portion.

161. The method of claim 160, wherein the 5' RNA portion of the amplification primer is adjacent to the 3' DNA portion.

162. The method of claim 149, wherein the RNA portion of the first primer consists of 7 to about 20 nucleotides.

163. The method of claim 162, wherein the DNA portion of the first primer consists of about 5 to about 20 nucleotides.

164. The method of claim 163, wherein the RNA portion of the first primer consists of about 10 to about 20 nucleotides.

165. The method of claim 164, wherein the DNA portion of the first primer consists of about 7 to about 20 nucleotides.

166. The method of claim 149, wherein the first primer is selected from the group consisting of 5'-*GACGGAUGCGGUCU*dCdCdAdGdTdGdT-3 (SEQ ID NO:1); and 5'-*CGUAUUCUGACGACGUACUC*dTdCdAdGdCdCdT-3' (SEQ ID NO:2), wherein italics denote ribonucleotides and "d" denotes deoxyribonucleotides.

167. The method of claim 149, wherein the reaction mixture further comprises a non-canonical nucleotide.

168. The method of claim 167, wherein the non-canonical nucleotide is dUTP.

169. The method of claim 149, wherein the reaction mixture further comprises a labeled nucleotide.

170. A method of making a polynucleotide array, comprising:
immobilizing polynucleotide amplification product onto a substrate, said polynucleotide amplification product produced according to any of methods of claims 1, 49, 65, 84, 108 or 149.

171. The method of claim 170, wherein said polynucleotide amplification products are generated by amplification of template polynucleotide from a defined source.

172. The method of claim 171, wherein the defined source is a defined cell population.

173. The method of claim 170, wherein said substrate is selected from the group consisting of paper, glass, plastic, nitrocellulose, silicon, and optical fiber.

174. The method of claim 170, wherein the substrate is a particle.

175. The method of claim 174, wherein the particle is a bead.

176. The method of claim 175, wherein the bead is labeled with a dye.

177. A method of characterizing a nucleic acid, comprising:
analyzing polynucleotide amplification product, said amplification product produced by the method of any of claims 1, 29, 65, 84, 108, or 149.

178. The method of claim 177, wherein the analyzing is carried out by contacting the amplification product with a probe.

179. The method of claim 177, wherein the analyzing is carried out by quantifying a sequence of interest in the amplification product.

180. The method of claim 177, wherein the analyzing is carried out by sequencing the amplification product.

181. The method of claim 177, wherein the analyzing is carried out by detecting any alteration in a target nucleic acid sequence in the amplification product, as compared to a reference nucleic acid sequence.

182. The method of claim 181, wherein detection of an alteration in a target nucleic acid sequence is carried out by a method selected from the group consisting of allele specific primer extension, allele specific probe ligation, differential probe hybridization, and limited primer extension.

183. A method for determining a gene expression profile, comprising:
quantifying amplification products of sequences of interest; wherein said amplification products are produced by any of the methods of claims 1, 29, 65, 84, 108, or 149.

184. The method of claim 183, wherein said amplification products are amplified from RNA template.

185. A method of preparing a library, comprising:
preparing a library of polynucleotide amplification products, said amplification products produced by any of the methods of claims 1, 29, 65, 84, 108, or 149.

186. A method for performing subtractive hybridization, comprising:
hybridizing a polynucleotide amplification product comprising multiple copies of target polynucleotide to a polynucleotide population, whereby a subpopulation of the polynucleotide population forms a complex with the polynucleotide amplification product, wherein said polynucleotide amplification product is produced according to the method of any of claims 1, 29, 65, 84, 108, or 149.

187. A method for differential amplification, comprising:
hybridizing a DNA driver polynucleotide population to a RNA population, whereby a subpopulation of the RNA population forms a complex with the driver population;

cleaving RNA with an agent that cleaves RNA in an RNA/DNA heteroduplex; and
amplifying the remaining RNA population according to the method of any of claims 1, 29, 65, 84, 108, or 149.

188. A method for archiving polynucleotide templates, comprising:
storing polynucleotide amplification product, wherein said polynucleotide amplification product is produced according to the method of any of claims 1, 29, 65, 84, 108, or 149.

189. A kit for amplifying template polynucleotide, said kit comprising:
a composite primer that is capable of binding to multiple sites within template polynucleotide; and

instructions for carrying out the method according to any of claims 1, 29, 65, 84, 108, or 149.

190. The kit of claim 189, further comprising auxiliary primers.